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Gas exchange and millet phytomass under organic fertilization and graywater irrigation[1]

Trocas gasosas e fitomassa do milheto sob adubação orgânica e irrigado com água cinza

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HIGHLIGHTS:

Higher gas exchange values during the initial growth stage are associated with significant phytomass accumulation in pearl millet.
Graywater did not provide enough nutrients to boost the millet phytomass accumulation.
Fertilization with bovine manure favored an increase in basal tiller mass.

ABSTRACT: Graywater is an alternative method to increase the water supply for agricultural production in semi-arid regions. The objective of this study was to evaluate the effects of different irrigation depths of graywater on the gas exchanges and phytomass of millet plants with and without organic fertilization. The research was conducted under greenhouse conditions in Serra Talhada municipality in semiarid region of Brazil, in a randomized complete block design with a factorial (4 × 2 + 1) plot and three replicates. The first factor corresponded to graywater irrigation depth equivalent to 25, 50, 75 and 100% of the available water content of the soil, and the second factor was the addition of bovine manure as fertilizer (0 and 34 Mg ha^{-1}), and a control (irrigation with low-salinity water). Irrigation with graywater effluent did not promote adverse effects on gas exchanges and phytomass accumulation; however, it also did not provide enough nutrients to promote increase in these variables. The reduction in irrigation depth caused a decrease in gas exchange from 45 days after the application of the treatments. The basal tiller mass was the most favored plant component due to organic fertilization.

Key words: *Pennisetum glaucum*, irrigation water depths, bovine manure, wastewater

RESUMO: A água cinza é um método alternativo para aumentar a oferta hídrica para produção agrícola nas regiões semiáridas. O objetivo deste estudo foi avaliar os efeitos de diferentes lâminas de irrigação de água cinza nas trocas gasosas e acúmulo de fitomassa do milheto com e sem adubação orgânica. A pesquisa foi conduzida em condições de casa de vegetação em Serra Talhada-PE, municipio na região semiárida do Brasil, em delineamento de blocos casualizados em esquema fatorial (4 × 2 + 1) e três repetições. O primeiro fator correspondeu à lâmina de irrigação com água cinza equivalente a 25, 50, 75 e 100% de água disponível do solo, e o segundo fator a adição de esterco bovino como fertilizante (0 e 34 Mg ha^{-1}), e um controle (irrigação com água de baixa salinidade). A irrigação com efluente de água cinza não promove efeitos negativos nas trocas gasosas e acúmulo de fitomassa, contudo, também não disponibiliza nutrientes suficientes para incrementar essas variáveis. A redução das lâminas de irrigação causou diminuição das trocas gasosas a partir dos 45 dias após aplicação dos tratamentos. A massa de perfilhos basais foi o componente da planta mais favorecida pela adubação orgânica.

Palavras-chave: *Pennisetum glaucum*, lâminas de irrigação, esterco bovino, água residuária

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Introduction

The irregularity of rainfall is the main factor limiting agricultural and livestock production in semi-arid regions. The use of wastewater for irrigation is a management strategy aimed to minimize the impact of water scarcity (Leonel & Tonetti, 2021). In addition, it is a source of nutrients (Schaer et al., 2014). Among the wastewater types, graywater effluent is useful for agriculture as it does not receive waste from latrines; thus, it has a low content of organic matter (less risk of clogging) and pathogenic organisms (less chance of contamination) (Leong et al., 2017).

The amount of water applied via irrigation to crops also plays a significant role in optimizing water resources (Ismail et al., 2018). However, a decrease in the volume of water applied to crops can cause disturbances in physiology (stomatal closure) and metabolism (accumulation of compatible osmolytes and production of reactive oxygen species [ROS]) in plants (Zhang et al., 2013; Chaves et al., 2016). These alterations reduce CO_2 uptake, slow down the Calvin cycle and interfere with plant growth (Liu et al., 2020).

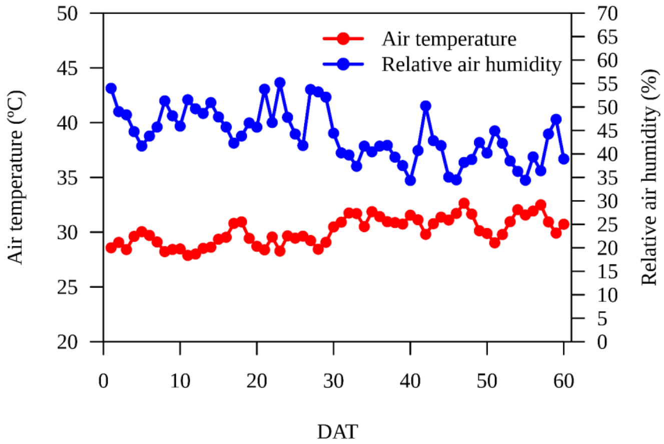
Millet [*Pennisetum glaucum* (L.) R. Br.] is characterized by adaptation to regions with low precipitation and high air temperatures (Nicolau Sobrinho et al., 2009), demonstrating that it is a promising species for cultivation in places receiving wastewater. Millet can replace maize and sorghum in animal diets, with these latter species more demanding of water and nutrients than millet (Brunette et al., 2016).

Although wastewater contains dissolved nutrients, it does not provide adequate nutrients (Santos Júnior et al., 2015). The high costs of industrialized fertilizers and their effect on the environment have encouraged organic fertilizer research, which can improve the physical, chemical, and biological characteristics of the soil (Vimal et al., 2017). The objective of the present study was to investigate the effects of different water regimes of wastewater (graywater effluent), on the gas exchange and accumulation of millet phytomass with and without organic fertilization.

Material and Methods

The experiment was conducted from September to November 2017, in a protected environment (greenhouse) at the Academic Unit of Serra Talhada, Universidade Federal Rural de Pernambuco, in the Brazilian semi-arid region (altitude: 429 m, latitude: 7°56'15" S and longitude: 38°18'45" W). According to Köppen's classification, the climate pattern of the region is semi-arid, hot, and dry (BShw). The environmental conditions (daily averages) of the greenhouse during the experiment presented a mean air temperature of 30.09 ± 1.57 °C and a mean relative air humidity of 44.00 ± 5.48% (Figure 1).

A randomized block design was adopted, in the factorial scheme 4 × 2 + 1 (control) with three replicates. The first factor was the irrigation depths with graywater effluent (25, 50, 75 and 100% of the available soil water [ASW]) and the second factor was organic fertilization (with and without application of bovine manure). The control received irrigation from the low-



DAT - Days after application of treatments

Figure 1. Dynamics of air temperature and relative air humidity in the greenhouse during the experimental period

salinity urban supply (0.20 dS m^{-1}) (chemical characteristics in Table 1) with an irrigation depth equivalent to 100% ASW and did not receive any fertilization.

The graywater effluent was collected from a rural residence in Carnaíba, Pernambuco State, Brazil (chemical characteristics in Table 1), and originated from a system that captured and filtered water from the bathroom, kitchen, and laundry washing of the family's residence. The filtration system consisted of two parts: a tank operated as a grease trap and a filter system formed by a superficial layer of charcoal and layers of coarse gravel, coarse sand, fine sand, and fine gravel that retained the larger fat particles, soap remnants, and organic materials in suspension that were not caught by the grease trap. Second, a tranquilizer tank where the water used for irrigation was captured.

The IPA-Buck-1 BF (*P. glaucum*) millet cultivar was sown in pots with a capacity of 18 dm^3, which were filled with soil until reaching a bulk density of 1.30 kg dm^{-3}. The soil was collected in the first 20 cm layer of a eutrophic Ta Haplic Cambisol and was sieved through a 4 mm mesh. The chemical and physical characteristics of the soil were 40.0 mg dm^{-3} of phosphorus, 43 mg dm^{-3} of iron, 0.68 cmol$_c$ dm^{-3} of potassium, 1.30 cmol$_c$ dm^{-3} of calcium, 0.27 cmol$_c$ dm^{-3} sodium, 1.0 cmol$_c$ dm^{-3} of hydrogen + aluminum, 0.88 dag kg^{-1} organic matter, 72.2% sand, 17.2% silt, and 10.5% clay, with pH of 7.1.

Bovine manure was added to the soil and homogenized for subsequent filling of the pots, with 645 g of manure added to

Table 1. Chemical analysis of graywater effluent (WW) and the urban supply water (WS) used in irrigation of millet

Element	WW	WS
Calcium (mmol$_c$ L^{-1})	0.64	2.24
Magnesium (mmol$_c$ L^{-1})	0.48	0.78
Sodium (mmol$_c$ L^{-1})	0.32	15.4
Potassium (mmol$_c$ L^{-1})	0.07	0.67
Carbonate (mmol$_c$ L^{-1})	0.00	0.12
Bicarbonate (mmol$_c$ L^{-1})	0.40	3.80
Sulfates (mmol$_c$ L^{-1})	0.04	0.29
Chloride (mmol$_c$ L^{-1})	0.60	6.12
Copper (mg L^{-1})	0.04	0.06
Iron (mg L^{-1})	0.08	0.08
Manganese (mg L^{-1})	0.03	0.05
Zinc (mg L^{-1})	0.05	0.05
pH	7.20	7.75
Electrical conductivity (dS m^{-1})	0.20	0.94

each pot, equivalent to a dose of 34 Mg ha^{-1}, which according to Nicolau Sobrinho et al. (2009), is sufficient for the total growth of millet. The chemical characteristics of the bovine manure were 10.44 g kg^{-1} of nitrogen, 5.28 g kg^{-1} of phosphorus, 10.50 g kg^{-1} of potassium, 11.20 g kg^{-1} of calcium, 6.85 g kg^{-1} of magnesium, 113.29 g kg^{-1} of carbon, and carbon: nitrogen ratio of 11. The treatments did not receive mineral fertilization.

Soil moisture at field capacity (FC) was determined in the pots following the methodology described by Casaroli & Jong van Lier (2008) to estimate the ASW. The moisture content at the permanent wilting point (PWP) was determined using undisturbed soil samples subjected to 15 atm in a Richards extraction chamber. The ASW was measured by subtracting the PWP from the FC moisture content. FC and PWP for soil without bovine manure were 0.18 and 0.03 g g^{-1}, respectively, and were 0.20 and 0.05 g g^{-1}, respectively, for soil with bovine manure.

Nine seeds were distributed per pot and placed at 2.0 cm depth. The stand formation period lasted until 15 days after crop emergence. All pots were irrigated daily using the urban supply to maintain the soil at FC. After this period, thinning was performed, leaving only one plant per pot. Then the different graywater effluent depths began based on the ASW (25, 50, 75, and 100% ASW). Irrigation occurred daily to replace the water mass lost through evapotranspiration, which was estimated by weighing the vessels (Casaroli & Jong van Lier, 2008).

The experiment was conducted for 60 days after the beginning of the treatments. Every 15 days, after the application of the treatments, gas exchange at the leaf level was determined by measuring net CO_2 assimilation (A), stomatal conductance (gs), intercellular CO_2 concentration (Ci), and transpiration (E). For these determinations, a portable infrared gas analyzer (model Licor 6400XT) was used and was operated with artificial light (PAR) of 1000 μmol of photons m^{-2} s^{-1}, ambient CO_2 concentration (390 ± 3.2 μmol CO_2), and chamber temperature of 28 °C. Observations were taken on the second fully expanded leaf, from top to bottom, between 8:00 and 11:00 a.m. after irrigation.

Using the gas exchange parameters, the instantaneous water use efficiency (A/E), intrinsic water use efficiency (A/gs), and instantaneous carboxylation efficiency (A/Ci) were estimated. Simultaneous with the gas exchange reading, the apparent electron transport rate (ETR) and the ratio between the ETR and net photosynthetic rate (ETR/A) were measured using a Licor 6400-40 modular fluorimeter.

At the end of the experiment, the following morphological components were collected and separated from the main tillers (largest growing tillers) and the basal tillers of the plants: live leaves, dead leaves, stem, and panicle. The material was dried in a forced-air ventilated oven at 65 °C until it reached a constant weight to determine the dry matter content.

Initially, the data were analyzed using the Shapiro-Wilk and Cochran homoscedasticity tests. Analysis of variance was conducted using the F test ($p < 0.05$) and the Tukey test ($p < 0.05$) to compare means, after which significant regression analysis was performed for quantitative factors (water fractions available in the soil). To compare the control treatment with those irrigated with graywater effluent (with and without organic fertilization), the Dunnett test ($p < 0.05$) was applied. R software (R Core Team, 2017) was used for data processing, figure creation, and statistical analysis.

Results and Discussion

From evaluation of gas exchanges at 15, 30, 45, and 60 days after the application of treatments (DAT), there was no interaction between treatments (irrigation depths and fertilization) for the simple effect of organic fertilization with or without bovine manure (Table 2). The differences between the control and treatments with and without organic fertilization are shown in Table 2.

At 15 DAT, the net CO_2 assimilation (A) in the fertilized plants was 22 and 30% higher than that in plants without

Table 2. Gas exchange variables of millet at 15, 30, 45 and 60 days after the treatment application (DAT) with graywater effluent irrigation and organic fertilization (S_1 = 34 Mg ha^{-1} of bovine manure and S_2 = 0)

Treatment	A	gs	E	Ci	ETR	A/E	A/gs	A/Ci	ETR/A
15 DAT									
S_1	26.1* A	0.13^{ns} A	3.67^{ns} A	36.6* A	102.8* A	7.1* A	198.7* A	0.89* A	4.0^{ns} A
S_2	20.3^{ns} B	0.10^{ns} B	3.03^{ns} B	47.0* A	84.8^{ns} B	6.7* A	195.0* A	0.60* A	4.2^{ns} A
C_T	18.1	0.10	2.92	75.9	81.8	6.2	177.4	0.24	4.5
30 DAT									
S_1	25.9^{ns} A	0.13^{ns} A	4.64^{ns} A	34.7* A	104.7^{ns} A	5.6* A	199.6* A	0.85* A	4.0* A
S_2	28.3^{ns} A	0.15^{ns} A	5.28^{ns} A	50.8* A	113.8^{ns} A	5.5* A	187.8* A	0.60* A	4.0* A
C_T	21.5	0.13	4.18	82.2	100.0	5.1	171.4	0.30	4.7
45 DAT									
S_1	24.3^{ns} A	0.13^{ns} A	4.1^{ns} A	53.2* A	112.7^{ns} A	5.9* A	190.3* A	0.50^{ns} A	4.1* A
S_2	28.2* A	0.15^{ns} A	4.7^{ns} A	53.0* A	97.0^{ns} B	6.0* A	188.4* A	0.75* A	4.0* A
C_T	18.9	0.11	3.9	83.6	92.0	5.4	173.6 a	0.23	4.8
60 DAT									
S_1	15.2^{ns} B	0.08^{ns} B	2.2^{ns} B	66.9* A	73.8^{ns} B	6.6* A	185.3* A	0.50^{ns} B	5.0^{ns} A
S_2	22.2^{ns} A	0.11^{ns} A	3.2^{ns} A	54.2* A	96.8* A	6.8* A	189.9* A	0.70* A	4.4* B
C_T	16.4	0.09	2.6	80.5	83.4	6.0	175.9	0.50	5.1

Means followed by * indicate the significant difference in relation to the control, means followed by ns indicate that there are no significant differences compared to control (C_T) by Dunnett test (p> 0.05). Means followed by the same uppercase letter in a column do not differ significantly by the Tukey test (p> 0.05) between treatments with fertilization (S_1) and without fertilization (S_2). A - Net CO_2 assimilation (μmol CO_2 m^{-2} s^{-1}); gs - Stomatal condutance to H_2O (mol H_2O m^{-2} s^{-1}); E - Transpiration (mmol H_2O m^{-2} s^{-1}); Ci - Intercellular CO_2 concentration (μmol CO_2 m^{-2} s^{-1}); ETR - Electron transport rate (μmol electrons m^{-2} s^{-1}); A/E - Instantaneous water use efficiency (μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}); A/gs - Intrinsic water use efficiency (μmol CO_2 m^{-2} s^{-1}/mol H_2O m^{-2} s^{-1}); A/Ci - Carboxylation efficiency (μmol CO_2 m^{-2} s^{-1}/μmol CO_2 m^{-2} s^{-1}); ETR/A - Indicative of electron drift (μmol electrons m^{-2} s^{-1}/μmol CO_2 m^{-2} s^{-1})

fertilization and the control, respectively (Table 2). Bovine manure fertilization increased the amount of nutrients in the soil solution, including nitrogen. An increase in the nitrogen availability to the plant promotes greater CO_2 assimilation. Li et al. (2013) stated that nitrogen increased the chlorophyll content in the leaves and activity of the chloroplasts.

The A did not differ between the control treatment and those irrigated with graywater for the other evaluation times (30, 45, and 60 DAT). At 30 and 45 DAT, A did not differ between treatments with and without fertilizer; however, a difference did occur at 60 DAT when A was higher in the treatment without fertilizer (Table 2). The A was higher when millet was in a balanced nutritional management, contributing to mitigating water stress and providing rapid initial growth (Kuwahara et al., 2016). By obtaining higher gas exchanges at the beginning of growth, plants that received fertilization completed their cycle in a shorter period than plants without fertilization, justifying the drop in gas exchanges they exhibited at 60 DAT.

The stomatal conductance (gs) and transpiration (E) followed the same pattern as A when comparing the treatments with and without fertilization and irrigated with graywater effluent (Table 2). When gs and E were compared between the control treatment and those irrigated with graywater effluent (with and without fertilization), no statistical differences were observed at any time points (Table 2).

The plants in the control treatment showed the lowest intrinsic efficiency of water use at all assessment times (Table 2), probably due to low A without decreasing gs by the same magnitude. This tendency was also observed for the instantaneous water use efficiency. A lower water use efficiency implies a more significant loss of water to fix 1 g of CO_2 (Comas et al., 2019).

At 15, 30, 45 and 60 DAT, the intercellular concentration of CO_2 (Ci) was higher in the control treatment than the treatments with and without organic fertilization (Table 2), which was possibly related to the lower A of the plants in the control treatment. Although A did not always differ between treatments, the A in the control treatment was always lower. The smaller the A, the greater the excess of CO_2 in the leaf mesophyll (because since CO_2 will not enter the Calvin cycle), consequently increasing the Ci (Liu et al., 2012).

The carboxylation efficiency (A/Ci) did not differ between treatments with and without fertilization at 15, 30, and 45 DAT. However, at 60 DAT, the A/Ci was higher in plants without fertilization. Therefore, the sharp drop in A at 60 DAT was probably related to the accelerated growth that millet had during the initial phase when fertilized, justifying the lower A/Ci found at 60 DAT. The control had lower A/Ci at 15 and 30 DAT; however, it was equal to the treatment without fertilization at 45 and 60 DAT, similar to the treatment with fertilization.

Most of the time, when A/Ci decreased, there was an increase in Ci. This increase in CO_2 in the mesophyll indicated that the substrate for photosynthesis was available in the plant; however, the plant could not enter the Calvin cycle. Thus, as there were no limitations in the entry of CO_2 into the leaf, the losses in A for the control treatment were associated with limitations in the photochemical phase (decrease in the supply of NADPH and ATP) or biochemical factors (regeneration and Rubisco carboxylation) (Galmés et al., 2011; Mashilo et al., 2017).

The reduction in A/Ci increases the susceptibility to photochemical damage, because there is an excess of light energy at the photosystem II level due to low CO_2 assimilation rates (Caemmerer & Furbank, 2016). This was confirmed by the increasing ETR/A (dynamics similar to carboxylation efficiency), indicating electron deviation for other acceptors that were not A. These free electrons could bind with oxygen, forming reactive oxygen species, causing oxidative damage to plant cells, thus affecting plant metabolism (Mittler, 2002).

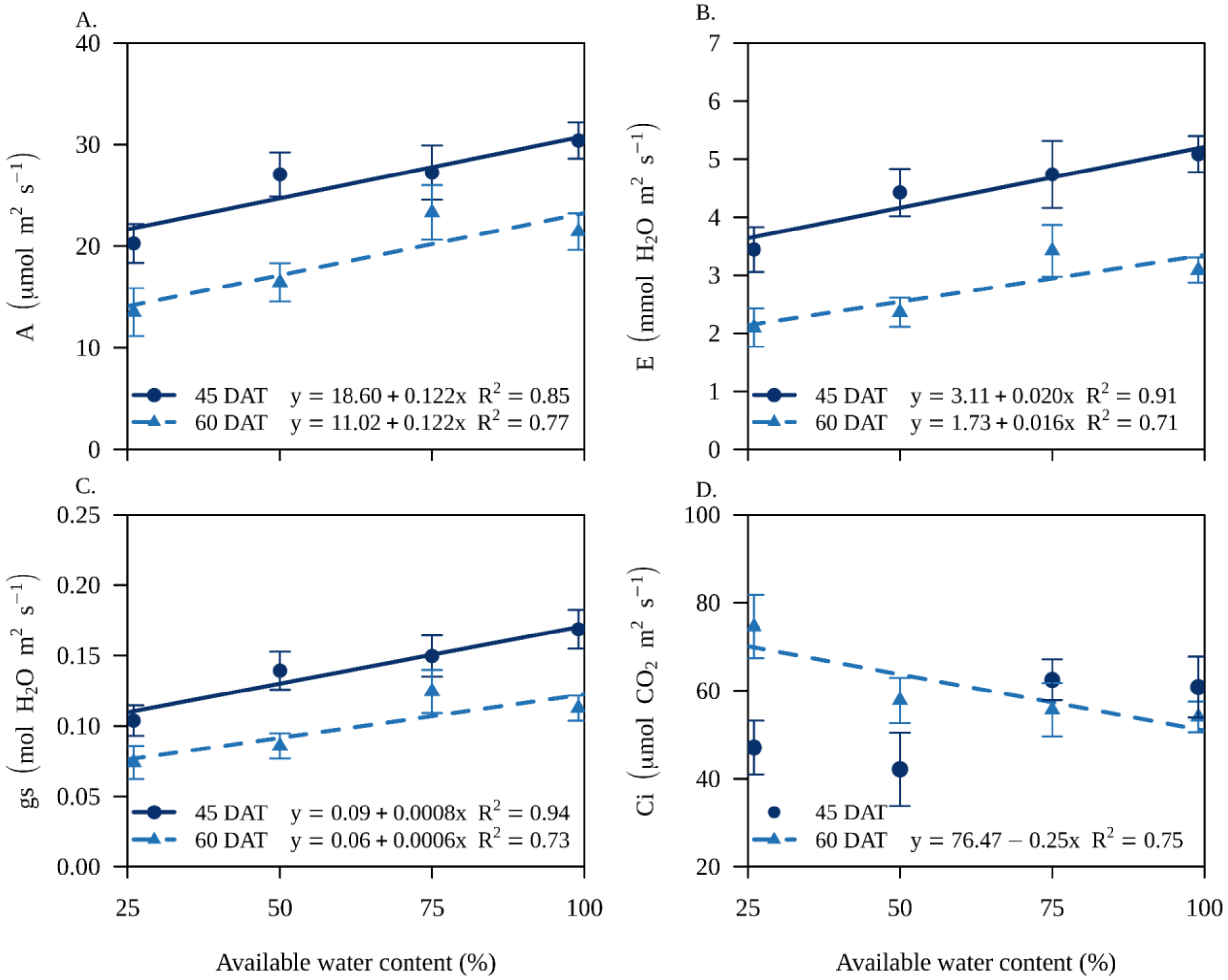
The effects of the different irrigation levels were observed only at 45 and 60 DAT, indicating the tolerance of millet to water stress during early growth stages. The high frequency of irrigation (daily) might have compensated for the effect of water deficit on gas exchanges, aiding in the tolerance of millet during the early growth stages. Gas exchanges are affected more intensely when long irrigation intervals are adopted (Puértolas et al., 2020). When the plants reached the stages that demanded more water (after 30 DAT), the frequency of irrigation and intrinsic tolerance of the crop were not adequate for overcoming the effect of deficit irrigation on gas exchange.

As the irrigation levels increased at 45 and 60 DAT, the A, E, and gs also increased (Figures 2A, B, and C). Irrigation with 25% ASW promoted a reduction of 30 and 38% in A at 45 and 60 DAT, respectively (Figure 2A). Under low water availability, plants control their stomatal opening (reduction of gas exchanges) to decrease water loss, resulting in a reduction in E and A, from which a cascade of effects on plant metabolism is initiated (Liu et al., 2012; Devi & Reddy, 2020). These metabolic changes depend on the intensity of stress and the number of stressful events (Singh et al., 2021).

The E was reduced by 29 and 36% when millet was irrigated with 25% ASW at 45 and 60 DAT, respectively (Figure 2B). In addition, a 35% reduction in gs was observed when millet was irrigated with 25% ASW at 45 and 60 DAT (Figure 2C). Thus, plants exposed to water shortage conditions, first responded to maintain water status closure of the stomata, resulting in a reduction in stomatal conductance and transpiration (Chaves et al., 2016).

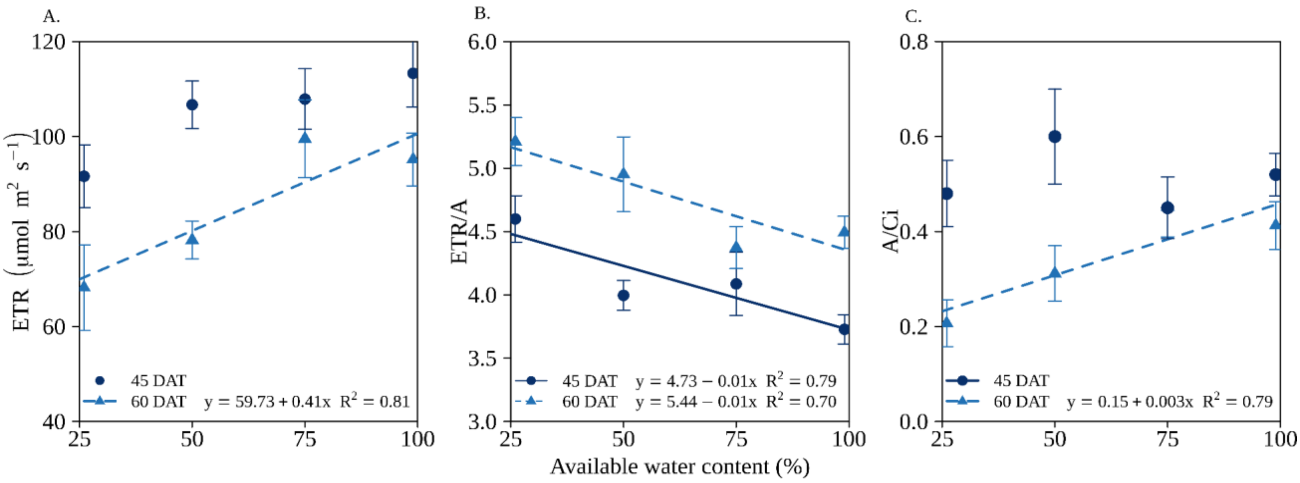
Regardless of irrigation levels, the Ci in the leaf mesophyll did not fit any model satisfactorily at 45 DAT, and as water availability in the soil decreased at 60 DAT, Ci increased (Figure 2D). The increase in Ci under water scarcity indicates that the reduction in photosynthesis was not only caused by stomatal regulation (Liu et al., 2012). Thus, the reduction in A value with increased water deficiency (Figure 2A) was also due to dysfunctions in the levels of biochemical reactions associated with CO_2 fixation, possibly due to limitations in the Rubisco synthesis caused by ATP deficiency (Mashilo et al., 2017).

The most drastic reduction in A was observed in the treatments with water deficiency at 60 DAT (Figure 2A) and was related to a decrease in the ETR (Figure 3A). The electron transport capacity is one of the main limiting mechanisms of CO_2 assimilation (Caemmerer & Furbank, 2016). The inability to transport electrons to supply reducers and ATP limits



The dots indicate the mean values measured with the respective standard deviation, and the lines indicate the values estimated by the regression

Figure 2. Gas exchanges of millet irrigated with different levels of wastewater (graywater effluent) at 45 and 60 days after application of the treatment (DAT): net CO_2 assimilation - A (A), transpiration rate - E (B), stomatal conductance - gs (C) and intercellular CO_2 concentration - Ci (D)



The dots indicate the mean values measured with the respective standard deviation, and the lines indicate the values estimated by the regression

Figure 3. Effect of available water content in the electron transport rate (ETR) (A); indicative of electron drift (ETR/A) (B) and, carboxylation efficiency (A/Ci) (C) of millet plants at 45 and 60 days after application of the treatment (DAT) irrigated with different levels of wastewater (graywater effluent)

Rubisco's regeneration (Zhang et al., 2013). Consequently, the net CO_2 assimilation is affected. The reduction in irrigation depth at 45 and 60 DAT promoted an increase in the ETR/A (Figure 3B), indicating a surplus of electrons in the photosynthetic process. When these free electrons bind with O_2, they form reactive oxygen species (ROS), promoting oxidative damage in plant cells (Mashilo et al., 2017). ROS production in thylakoids can also cause the deactivation of enzymes related to photosynthesis and inhibition of the functional activity of photosystem II (Zhang et al., 2013).

The carboxylation efficiency (A/Ci) was affected at 60 DAT due to the ASW, which increased proportionally with the irrigation levels (Figure 3C). The irrigation water regime did not influence the intrinsic and instantaneous water use efficiency due to the decrease in A, gs, and E being in the same magnitude as a function of the irrigation depth. Water use efficiency is an important mechanism of physiological adaptation, which can improve crop productivity under limited water availability (Bhattarai et al., 2020), and plants regulate their metabolism to continue assimilating CO_2, with restrictions on transpiration.

The phytomass production (dry matter) of millet plants, regardless of the available water level in the soil, was higher for all fertilization treatments (Table 3). Therefore, graywater contains nutrients that cannot increase millet production. The amount of nutrients in the graywater effluent used in the experiment was related to the natural fertility of the soil used, which does not include inputs from residential latrines that, generally contribute to a large part of the nutrients in wastewater (Larsen & Lienert, 2001; Santos Júnior et al., 2015). Human urine can represent more than 80% of the nitrogen found in domestic effluent, 50% of the phosphorus load, 90% of the potassium load, and less than 1% of the total volume of conventional domestic effluent (Larsen & Lienert, 2001).

According to Santos Júnior et al. (2015), the addition of 4.5% human urine to domestic effluent applied via irrigation in millet plants, resulted in dry mass and water use efficiency similar to those observed in plants under mineral fertilization and irrigation with municipal supply water. However, when human waste is present in wastewater, more advanced treatment techniques are required to avoid the risk of environmental and human contamination. This entails a higher cost for small farmers, which can make the reuse of wastewater economically unfeasible.

The additional cost of bovine manure fertilization is justified by increased production because since millet production was increased by more than 100% regardless of the irrigation level, when fertilized with bovine manure (Table 3). The production of millet plants irrigated with graywater effluent and without fertilization was not influenced by the irrigation regimes (Table 3). These same treatments did not differ from the control, which exhibited a dry matter production of 15.39 g plant^{-1}.

However, plants fertilized and irrigated with 25% ASW differed from other treatments with fertilization, i.e., the phytomass production in the 50, 75 and 100% ASW treatments were 66, 53 and 63% higher than that in the 25% ASW treatments respectively (Table 3). Bovine manure fertilization accelerated the vegetative growth of millet during the early growth stages. However, when plants are exposed to water deficit, they suffer more from this stress because they have a more developed leaf mass, requiring more water (Uppal et al., 2015).

Comparison of the phytomass accumulation of plants irrigated with graywater effluent and fertilized in the control treatment revealed increases of 102, 236, 209, 230 for 25, 50, 75, and 100% ASW, respectively. These results highlight the importance of organic fertilization for millet to achieve higher yields; even under the effect of water stress (25% ASW), the accumulation of phytomass was greater than that of the control.

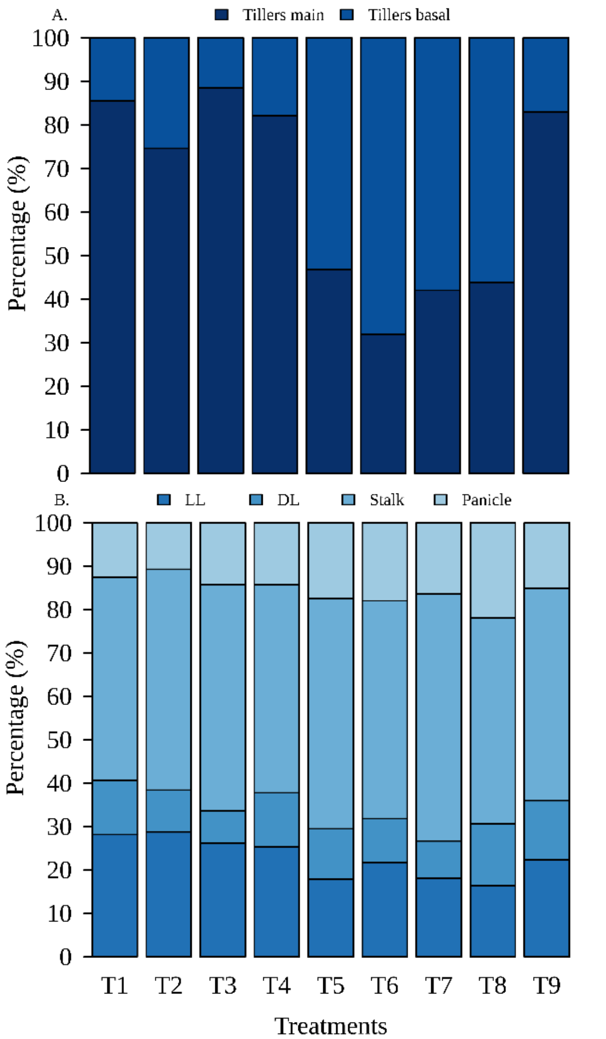
At 45 and 60 DAT, A increased linearly with ASW (Figure 3A); however, the millet phytomass at 50, 75, and 100% ASW did not differ significantly. The frequency of irrigation directly influenced the biomass productivity of plants, and the shorter the time interval between the water application events, the lower the effect of the water deficit (Puértolas et al., 2020).

Organic fertilization benefited the dry mass of the basal tillers more than the mass of the principal tillers (Figure 4A).

Table 3. Total dry phytomass yield of millet irrigated with wastewater (graywater effluent), without and with organic fertilization (0 and 34 Mg ha^{-1} of bovine manure)

Organic fertilization	Available water soil - AWS (%)				Control treatment
	25	50	75	100	
	(g MS plant^{-1})				
With (S_1)	31.22 bA*	51.85 aA*	47.65aA*	50.80 aA*	15.39
Without (S_2)	12.52 aB^{ns}	15.67 aB^{ns}	17.49 aB^{ns}	15.31 aB^{ns}	

Means followed by the same lowercase letter in row and uppercase letter in column do not differ significantly at 0.05 probability level by the Tukey test. Means followed by * indicate the significant difference in comparison to the control, means followed by ns indicate that there are no differences in comparison to the control by Dunnett test ($p < 0.05$)



T_1 - 100% available water in soil (AWS) without manure; T_2 - 75% AWS without manure; T_3 - 50% AWS without manure; T_4 - 25% AWS without manure; T_5 - 100% AWS with manure; T_6 - 75% AWS with manure; T_7 - 50% AWS with manure; T_8 - 25% AWS with manure; T_9 - Control treatment

Figure 4. Partition of millet dry matter in main tillers and basal tillers (A) and plant partition in live leaves (LL), dead leaves (DL), stalk and panicle of millet plants (B) irrigated with graywater effluent and without and with organic fertilization (0 and 34 Mg ha^{-1} of bovine manure)

In fertilized treatments, the dry mass of basal tillers contributed to more than 50% of the total dry mass of the plant, whereas in the unfertilized treatments it contributed only 15% of the total dry mass. The proportion of live leaves was lower in the fertilized treatments (Figure 4B). Thus, the treatments that received more water and fertilization showed accelerated growth, as indicated by gas exchange (Table 2); consequently, a greater mass of dead leaves was observed when harvested in the advanced growth stages.

The fertilized treatments showed panicles in the basal tillers, contributing to the higher proportion of panicles in the dry mass of plants (Figure 4B), thus causing a better quality of forage, as the panicles have high starch content in the grains. The increase in the proportion of panicles is important to produce silage and higher amounts of organic acids (notably lactic acid), and increase the quality of millet silage (Brunette et al., 2016). The phytomass partition of the control treatment was similar to that of the treatment irrigated with 100% AWS of graywater effluent with no fertilization (Figure 4). This result highlighted that graywater did not promote harmful effects on millet production; further, it also did not increase the phytomass partition.

Conclusions

1. Wastewater (graywater effluent) did not negatively influence the phytomass production of millet. However, fertilization was required to achieve a high phytomass yield.

2. Irrigation with graywater effluent promoted an increase in carboxylation efficiency and intrinsic water use efficiency. While the fertilization with organic bovine manure favored an increase in gas exchange at 15 DAT.

3. The reduction in gas exchange after 45 DAT decreased the accumulation of millet phytomass in the fertilized plants and those irrigated with 25% of the available soil water.

4. The application of 50% of the available water to the soil fertilized with bovine manure promoted a more significant water saving without reducing phytomass accumulation.

5. The basal tillers mass of millet was the component most favored by organic fertilization.

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